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A COMPARISON OF THE CUP-PLATE AND SERIAL DILUTION METHODS OF PENICILLIN ASSAYS*

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It is generally conceded that microbiological procedures offer the most accurate assay methods for the determination of penicillin in the serum (1, 2, 3, 4, 5). Two of these are recognized as being acceptable for this purpose. One of these, the "cup-plate method", has as its end-point the inhibition of bacterial growth on a solid agar plate after the application of serum containing penicillin. This serum is placed on the plate in small cups under standard conditions. The zone of no growth is measured and calculations made which determine the unitage strength of the serum being tested. The organism of choice for this assay is Sarcina lutea. This method has been utilized extensively by us in studies reported previously (6, 7, 8).

The other procedure, the "serial dilution" method, has as its basis the inhibition of growth of penicillin sensitive organisms (B. subtilis) in a suitable liquid medium in the test tube. Serial dilutions of the serum of patients who have received penicillin are assayed directly for antibiotic activity. This method is likewise performed under standard conditions, and calculations are made to determine the unitage values of penicillin in the serum.

For several months we have employed both assay methods simultaneously in assaying the serum of patients. Results of these methods differed so widely on occasions that it was felt advisable to investigate the cause of the accuracy and reproducibility of the two methods.

Known standards of crystalline sodium penicillin G were prepared in three concentrations in each of three diluents in an effort to establish such differences as may obtain in various media. Chart I refers to individual assays performed by the two different methods utilizing gelatin, phosphate buffer, and human sera. As will be noted in the key appearing below the chart, the different assay methods are denoted by different symbols. The broken horizontal lines on the chart represent known concentrations of accurately weighed crystalline sodium penicillin G standard diluted with the three diluents. The squares are serial dilution test values and the straight line pattern indicates only that the serum inhibited at one dilution and not at the next higher dilution. This does not indicate unusual accuracy or consistency. The circles are cup-plate assay values. These are derived from actual readings in millimeters of a zone of inhibition of bacterial growth.

Fifteen duplicate assay values obtained by each method at each concentration with each diluent are shown. *All assays were performed immediately after the preparation of the standards from the dry stable salt.*

Table I presents numerical data showing the average blood level in units per cc obtained by these methods of assay in the testing of the various standards. The percentage of the standard measured by both methods is also recorded.

SUMMARY

Two different microbiological procedures were performed simultaneously in checking the amount of crystalline sodium penicillin G in known standards prepared in gelatin, phos-

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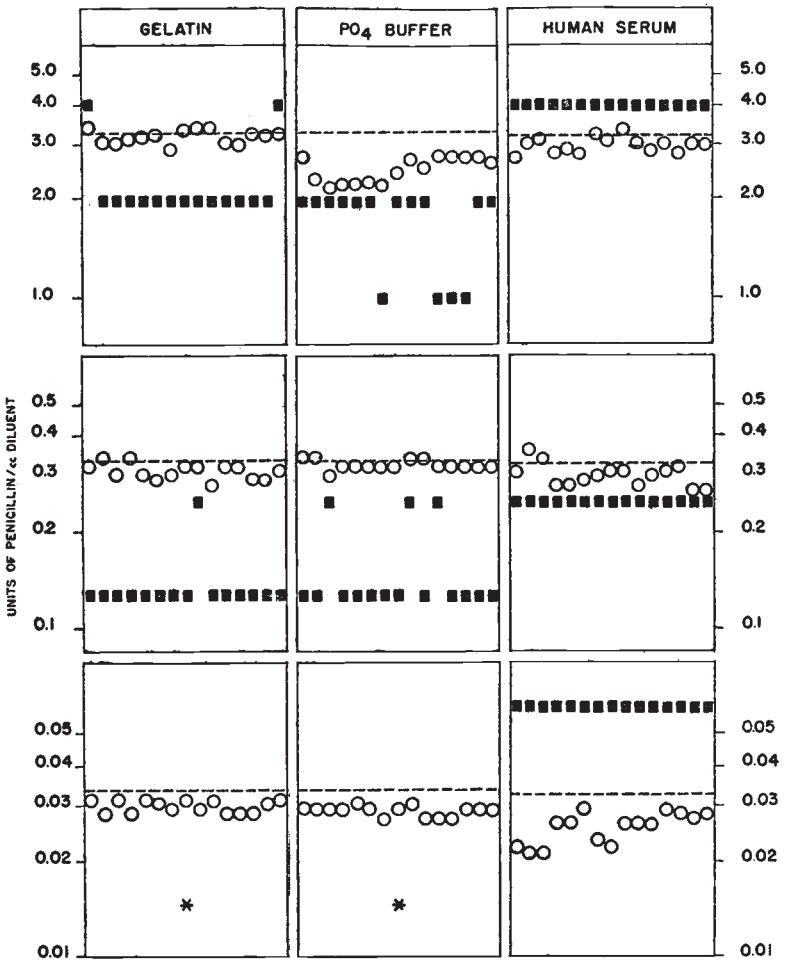
CHART No.I

PENICILLIN ASSAY COMPARISONS

DILUENTS



○ = CUP PLATE ASSAY – SARCINA LUTEA TEST ORGANISM.

■ = SERIAL DILUTION ASSAY – BACILLUS SUBTILIS TEST ORGANISM.

-- LINES INDICATE KNOWN STANDARD PENICILLIN CONCENTRATIONS.

* SERIAL DILUTION METHOD NOT SENSITIVE AT 0.033 CONCENTRATION IN GELATIN AND PHOSPHATE BUFFER.

TABLE I

Cup plate vs. serial dilution assay methods

(Calculations from 15 duplicate assays at each concentration)

	GELATIN		AQUEOUS PO_4 BUFFER		HUMAN SERUM	
	Cup Plate	Ser. Dil.	Cup Plate	Ser. Dil.	Cup Plate	Ser. Dil.
Known standard, units per cc.....	3.30	3.30	3.30	3.30	3.20	3.20
Aver. Blood Level, units per cc....	3.11	2.27	2.52	1.71	2.91	4.0
% of known standard measured....	94.6	69.3	76.2	51.8	91.0	133.0
Known standard, units per cc.....	0.33	0.33	0.33	0.33	0.32	0.32
Aver. blood level, units per cc.....	0.309	0.117	0.324	0.15	0.31	0.25
% of known standard measured....	91.4	35.5	98.2	45.5	97.0	78.0
Known standard, units per cc.....	0.033	0.033	0.033	0.033	0.032	0.032
Aver. blood level, units per cc.....	0.033	0	0.029	0	0.025	0.06
% of known standard measured....	100.0	—	87.0	0	79.5	187.0

phate buffer and human serum diluents. Under the conditions studied it is apparent that the cup-plate assay procedure is more accurate than the serial dilution method.

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